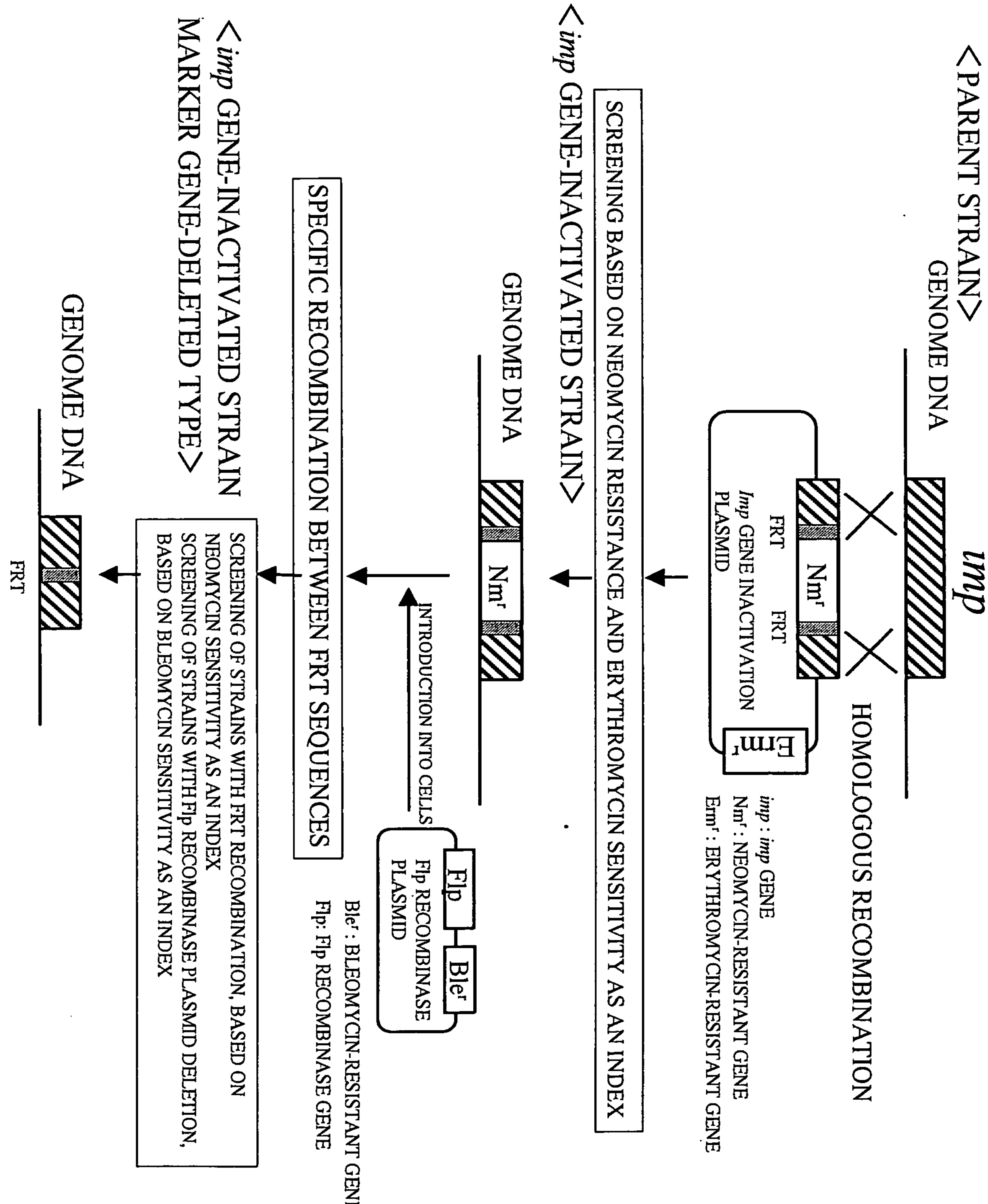
Fig.1


<PARENT STRAIN>
GENOME DNA
imp
HOMOLOGOUS RECOMBINATION
FRT
Nm^r
FRT
Erm^r
Imp GENE INACTIVATION PLASMID
imp : imp GENE
Nm^r : NEOMYCIN-RESISTANT GENE
Em^r : ERYTHROMYCIN-RESISTANT GENE
SCREENING BASED ON NEOMYCIN RESISTANCE AND ERYTHROMYCIN SENSITIVITY AS AN INDEX
<imp GENE-INACTIVATED STRAIN>
GENOME DNA
Nm^r
INTRODUCTION INTO CELLS
Flp
Ble^r
Flp RECOMBINASE PLASMID
Ble^r : BLEOMYCIN-RESISTANT GENE
Flp: Flp RECOMBINASE GENE
SPECIFIC RECOMBINATION BETWEEN FRT SEQUENCES
SCREENING OF STRAINS WITH FRT RECOMBINATION, BASED ON NEOMYCIN SENSITIVITY AS AN INDEX
SCREENING OF STRAINS WITH Flp RECOMBINASE PLASMID DELETION, BASED ON BLEOMYCIN SENSITIVITY AS AN INDEX
<imp GENE-INACTIVATED STRAIN
MARKER GENE-DELETED TYPE>
GENOME DNA
FRT

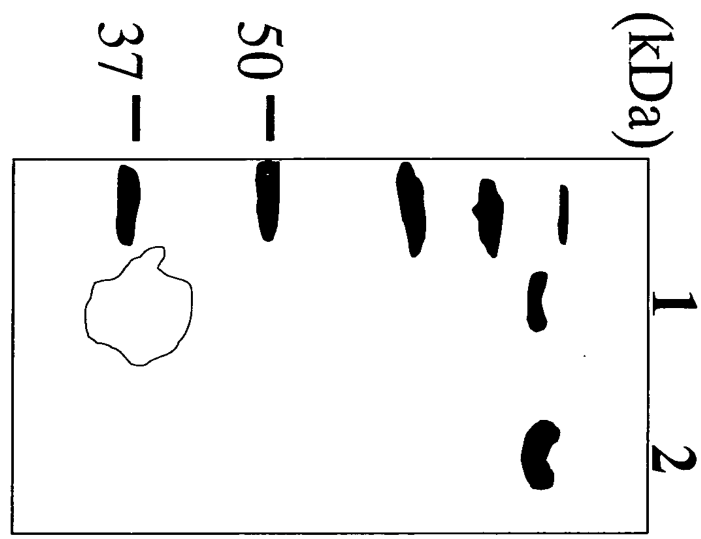


Fig. 2

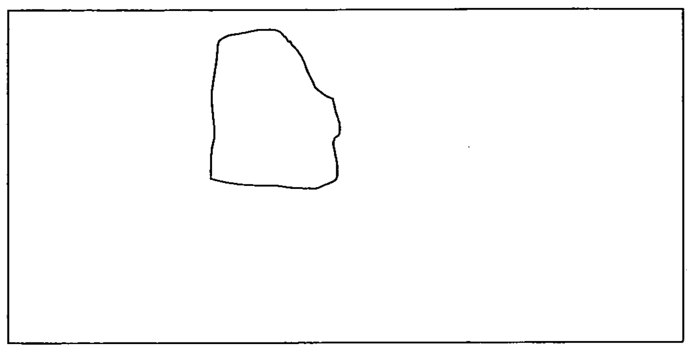


Fig. 3

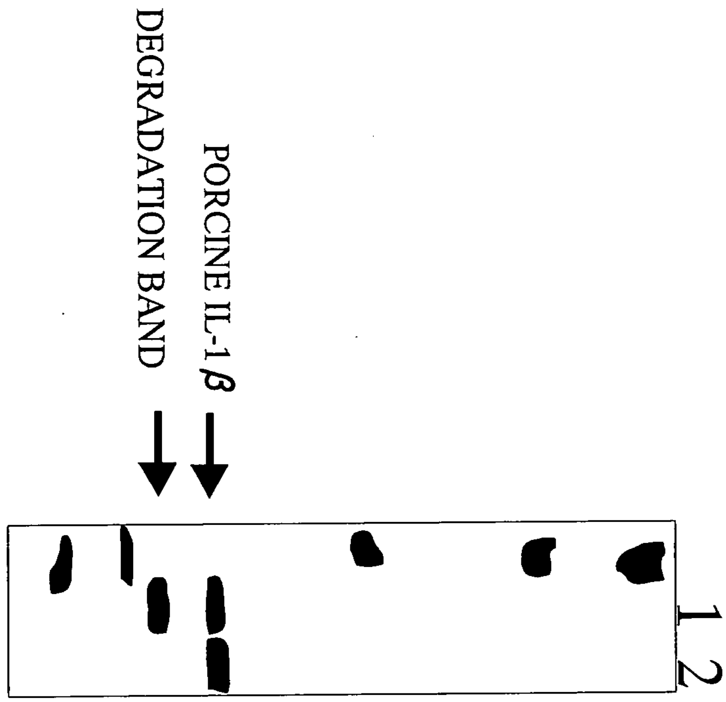


Fig. 4

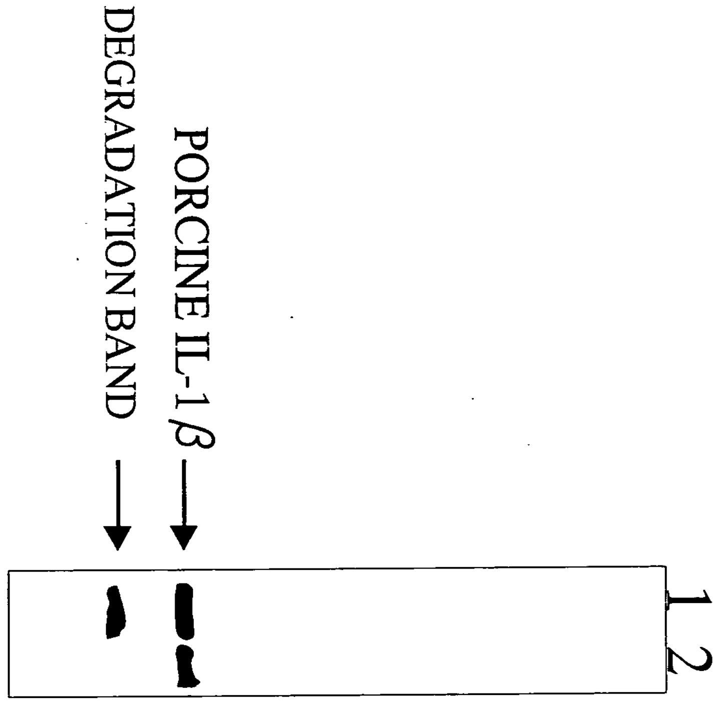


Fig. 5

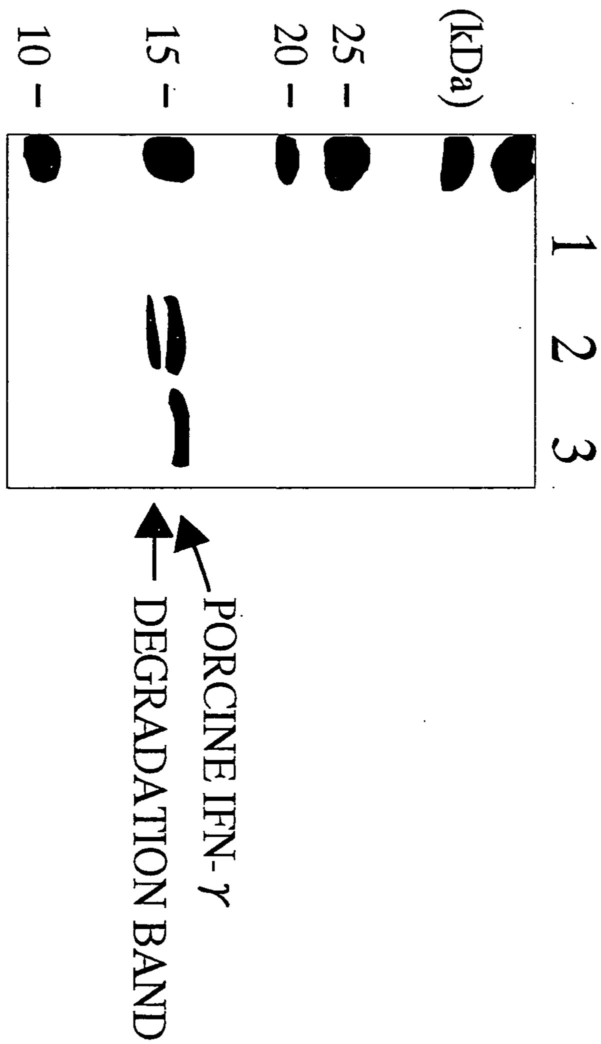


Fig. 6

Fig. 7

hos

1 ATGGGTGCCGATATCAAAAATGCGAGTCAACCATTTCTGACCAATGACCAAGTGAAAGAT 60
MetGlyAlaAspIleLysAsnAlaSerGlnProPheLeuThrAsnAspGlnValLysAsp

61 TTGATAGCCAAGAGCCAAGCTGGCGATACGGATGCACGTGAGCTTCTCGTGAATAGCAAT 120
LeuIleAlaLysSerGlnAlaGlyAspThrAspAlaArgGluLeuLeuValAsnSerAsn

121 ATCAGACTGGTCTGGTCCGTCGTCCAGCGCTTTATCAACCGCGGGTATGAAGCGGATGAT 180
IleArgLeuValTrpSerValValGlnArgPheIleAsnArgGlyTyrGluAlaAspAsp

181 TTGTTTCAGATCGGTTGCATTGGCTTGCTCAAGGCCGTTGACAAGTTCGATCTTTCGTAC 240
LeuPheGlnIleGlyCysIleGlyLeuLeuLysAlaValAspLysPheAspLeuSerTyr

241 GATGTGAGATTTTCGACCTATGCGGTGCCAATGATCATCGGAGAAATTCAACGCTTTTTG 300
AspValArgPheSerThrTyrAlaValProMetIleIleGlyGluIleGlnArgPheLeu

301 CGCGATGACGGTACGGTTAAGGTCAGTCGATCGTTAAAAGAAACAGCGAATAAGGTGCGG 360
ArgAspAspGlyThrValLysValSerArgSerLeuLysGluThrAlaAsnLysValArg

361 CGATCAAAGGATGAATTGTACAAGCAATTCGGCCGTGCCCCCACGATCGCAGAAGTGGCA 420
ArgSerLysAspGluLeuTyrLysGlnPheGlyArgAlaProThrIleAlaGluValAla

421 GAAGCAGTGGGAATCACGCCGGAGGAAGTAGTCTTTGCGCAAGAGGCAAGCAGAGCGCCT 480
GluAlaValGlyIleThrProGluGluValValPheAlaGlnGluAlaSerArgAlaPro

481 TCCTCCATCCATGAGACCGTTTTTGAAAATGACGGCGATCCCATCACACTGATCGATCAG 540
SerSerIleHisGluThrValPheGluAsnAspGlyAspProIleThrLeuIleAspGln

541 ATAGCGGATGAAGGTGTGAACAAGTGGTTTGAGAAAATTGCCTTGAAGGACGCCATCAGC 600
IleAlaAspGluGlyValAsnLysTrpPheGluLysIleAlaLeuLysAspAlaIleSer

601 AGGCTGAGCGAGCGTGAGCAGCTCATCGTCTACCTGCGCTATTACAAGGATCAGACACAG 660
ArgLeuSerGluArgGluGlnLeuIleValTyrLeuArgTyrTyrLysAspGlnThrGln

661 TCTGAGGTAGCAGAGCGTCTAGGGATTTCGCAGGTCCAGGTCTCGCGTCTGGAAAAGCGT 720
SerGluValAlaGluArgLeuGlyIleSerGlnValGlnValSerArgLeuGluLysArg

Fig. 8

721 ATCCTGCTAACGATCAAGGAGCAAATTGAACATTAG 756
IleLeuLeuThrIleLysGluGlnIleGluHis***

Fig. 9

emp

1 GTGAACGCAGTGAAGAAAGGCAAGAAGCTATTATCCATCCTATTTTCTTCCTCACTGGTC 60
ValAsnAlaValLysLysGlyLysLysLeuLeuSerIleLeuPheSerSerSerLeuVal

61 CTGAGCGGCATTGCGGCGGTTCCAGCGACAGGGATGGCCAAGTCAAAGGACAAGCCGCCG 120
LeuSerGlyIleAlaAlaValProAlaThrGlyMetAlaLysSerLysAspLysProPro

121 CTTGAAGTGGATTTGTCCACAGTGAACATGGATCGTTTGGTTAAAGCCTTGATCGACCAA 180
LeuGluValAspLeuSerThrValAsnMetAspArgLeuValLysAlaLeuIleAspGln

181 GGTGAAATCGACGAGGACGCCGACCAGGAAGAGATCAACAAAGCTGTGGAGAAGTTTTTG 240
GlyGluIleAspGluAspAlaAspGlnGluGluIleAsnLysAlaValGluLysPheLeu

241 AGAGACAAGAAAGTTCCCCACGGCATTGATGACTCCAGCTCCTTCGGGAAAAAAGCAAGC 300
ArgAspLysLysValProHisGlyIleAspAspSerSerSerPheGlyLysLysAlaSer

301 AAAACCCAGCTTTCGGCAGTATCAAAGGCAGCAAGCAAAGTATCCAAGCTCAAAGATGAC 360
LysThrGlnLeuSerAlaValSerLysAlaAlaSerLysValSerLysLeuLysAspAsp

361 AAGCAAGTGCGCGCTTCCAAGCGGGTACATACGGATAATCTGGTGATTGCCCTGGTCGAG 420
LysGlnValArgAlaSerLysArgValHisThrAspAsnLeuValIleAlaLeuValGlu

421 TTCAATGATCTGGAGCACAACCAGGTGCCAAAACAAAGCGATTCCTTGTGGACGGCAGAC 480
PheAsnAspLeuGluHisAsnGlnValProLysGlnSerAspSerLeuTrpThrAlaAsp

481 TTCGACCAAAAGCACTACGAGGAAATGCTGTTCGATCGTAAAGGCTATACGACTCCTGAA 540
PheAspGlnLysHisTyrGluGluMetLeuPheAspArgLysGlyTyrThrThrProGlu

541 GGGATAAGCATGACCACGATGGCCAAGTACTACTACGAGCAATCGGGTGAGACATGGACC 600
GlyIleSerMetThrThrMetAlaLysTyrTyrTyrGluGlnSerGlyGluThrTrpThr

601 GTGGATGGGGTTGTCACTCCGTGGTTGACTGCCGAAAAAGATAAGAAATTCTACGGTGGA 660
ValAspGlyValValThrProTrpLeuThrAlaGluLysAspLysLysPheTyrGlyGly

661 AACGATGAAAACGGCAACGATGCCAACCCACGCGATCTGGTCGTCGAGACACTGGAATCT 720
AsnAspGluAsnGlyAsnAspAlaAsnProArgAspLeuValValGluThrLeuGluSer

721 GTAGGGGATGCCATCAAGGGTCATGAAGAAGAATACGACCAACGCGACCCGTATGACTTG 780
ValGlyAspAlaIleLysGlyHisGluGluGluTyrAspGlnArgAspProTyrAspLeu

781 GATGGAGACAGCGATCTGATGGAGCCGGATGGCATGCTGGACAACCTGATGCTGGTTCAC 840
AspGlyAspSerAspLeuMetGluProAspGlyMetLeuAspAsnLeuMetLeuValHis

Fig. 10

841 TCCGGTATTGGTGAAGAGACTGGGGAAGATGCGGATGCGATCTGGTCTCACCGCTGGACT 900
SerGlyIleGlyGluGluThrGlyGluAspAlaAspAlaIleTrpSerHisArgTrpThr

901 CTGAAAAAGCCGACAGAAATTCCAGGCACCAGCCTGAAAGCTTACGACTACATGATTCAG 960
LeuLysLysProThrGluIleProGlyThrSerLeuLysAlaTyrAspTyrMetIleGln

961 CCTGAAGATGGCGCACCCGGCGTATTCGCACATGAATACGGACACAACCTGGGACTGCCA 1020
ProGluAspGlyAlaProGlyValPheAlaHisGluTyrGlyHisAsnLeuGlyLeuPro

1021 GATCTGTATGACACGACAAGACTGGGACATGATTCGCCGGTTGGCGCATGGTCGCTGATG 1080
AspLeuTyrAspThrThrArgLeuGlyHisAspSerProValGlyAlaTrpSerLeuMet

1081 TCTTCCGGAAGCCATACAGGTAAGATCTTCCAAACCCAACCAACCGGATTTGATCCTTGG 1140
SerSerGlySerHisThrGlyLysIlePheGlnThrGlnProThrGlyPheAspProTrp

1141 TCCAAAATGATGCTGCAGGAAATGTATGGGGGCAAGTGGATTGAGCCGCAAGTCATCAAT 1200
SerLysMetMetLeuGlnGluMetTyrGlyGlyLysTrpIleGluProGlnValIleAsn

1201 TACGAAGACCTGAAAAAACGGAAAAAGCAGGCTTCGCTCTACGATGGCAGCAGCCTCGAT 1260
TyrGluAspLeuLysLysArgLysLysGlnAlaSerLeuTyrAspGlySerSerLeuAsp

1261 GAAGATGGCAAAGTCATCAAGCTGAATATGCCGCAAGTAGAGAAGACACCGCCGGTTCAA 1320
GluAspGlyLysValIleLysLeuAsnMetProGlnValGluLysThrProProValGln

1321 CCGAAAGACGGCGATTATTCTTACTTCTCCGATGAGGGCGACAATCTGAACACGAAGATG 1380
ProLysAspGlyAspTyrSerTyrPheSerAspGluGlyAspAsnLeuAsnThrLysMet

1381 ACTTCGGAAGTGATCGACCTGACAGGCGCCAGCTCCGCATCGATGAGCTTCGACTCCTGG 1440
ThrSerGluValIleAspLeuThrGlyAlaSerSerAlaSerMetSerPheAspSerTrp

1441 AGAGCGATCGAGACCGGGTACGACTACCTGTACGTGAACGTGATTGATGTCGACTCAGGT 1500
ArgAlaIleGluThrGlyTyrAspTyrLeuTyrValAsnValIleAspValAspSerGly

1501 GAGAGCACAACAGTAAAAGAGTACGATGACGAAACCAAAGGCTGGGATAAGGAAGAAATC 1560
GluSerThrThrValLysGluTyrAspAspGluThrLysGlyTrpAspLysGluGluIle

1561 AGCCTGAACGATTTCGCTGGCAAAAAGATTCAAGTCGAGTTCAACTACGTGACGGATGGC 1620
SerLeuAsnAspPheAlaGlyLysLysIleGlnValGluPheAsnTyrValThrAspGly

1621 GGCTTGGCGATGTCCGGCTTCTATCTGGATAATTTTGCAGTCACAGCAGACGGCGAAGTA 1680
GlyLeuAlaMetSerGlyPheTyrLeuAspAsnPheAlaValThrAlaAspGlyGluVal

1681 GTCTTCTCGGATGATGCAGAAGGCGACCAGAAGTTTGATCTGGATGGATTCATCCATTTC 1740
ValPheSerAspAspAlaGluGlyAspGlnLysPheAspLeuAspGlyPheIleHisPhe

Fig. 11

```
1741  GACGGCGAAGGCAAAATGTACGACGCGTACTACCTGGTAGAGCTGCGCTCCCATGAAGGC  1800
      AspGlyGluGlyLysMetTyrAspAlaTyrTyrLeuValGluLeuArgSerHisGluGly

1801  GTGGACGAGGGTCTGAAATACTTCCGCCGCAATGACACATTCTTCACGTATGATCCAGGT  1860
      ValAspGluGlyLeuLysTyrPheArgArgAsnAspThrPhePheThrTyrAspProGly

1861  CTGGTGATCTGGTACTACGATGGACGCTTTGGCAAAACGCAAGACAACAACACCAGCAAC  1920
      LeuValIleTrpTyrTyrAspGlyArgPheGlyLysThrGlnAspAsnAsnThrSerAsn

1921  CATCCAGGCTACGGCATGCTGGGCGTAGTCGATGCGCATCAGGAAGTTCGTTACTGGAAT  1980
      HisProGlyTyrGlyMetLeuGlyValValAspAlaHisGlnGluValArgTyrTrpAsn

1981  AACGATGAGGGCAACGAGGAGGCCATTGCCGACTCCCGTTACCAAGTGAACGATGCGGCA  2040
      AsnAspGluGlyAsnGluGluAlaIleAlaAspSerArgTyrGlnValAsnAspAlaAla

2041  TTCAGCCCGAACAAAACCTCCGGCATGGATCTCGACTACATTCTCGGCACGATGGATTAC  2100
      PheSerProAsnLysThrSerGlyMetAspLeuAspTyrIleLeuGlyThrMetAspTyr

2101  GAGCCGCTGAAAGGCATTACCGTATTCAAAGACAGTGATGATTACACGATGCCGGAAGTT  2160
      GluProLeuLysGlyIleThrValPheLysAspSerAspAspTyrThrMetProGluVal

2161  CCGGAAATCGGAAAAATCCTGCCGAAGATCGGTCTGCAAATCAAATTAATTCGTGTGTCC  2220
      ProGluIleGlyLysIleLeuProLysIleGlyLeuGlnIleLysLeuIleArgValSer

2221  AAGAAATTCACGAACGCACAGGTCGAGTTCTCCATCAAAAAATAA  2265
      LysLysPheThrAsnAlaGlnValGluPheSerIleLysLys***
```

Fig. 12

imp

1 ATGAACCATCCTGATTTTCGCGATCTACCCGCCTGCATGGAAGACGTAACCCTCGCTGCC 60
MetAsnHisProAspPheArgAspLeuProAlaCysMetGluAspValThrLeuAlaAla

61 CTGGACGAGTACACTGGTCCACCAGATCCGACCGAATACCAATCATTGTATGGACGCTTG 120
LeuAspGluTyrThrGlyProProAspProThrGluTyrGlnSerLeuTyrGlyArgLeu

121 CAAGAGGTTGCCGAAACTCTCCCTCCGCTCTATCGGGAGCATGTGTATCACCCTTTTCTT 180
GlnGluValAlaGluThrLeuProProLeuTyrArgGluHisValTyrHisProPheLeu

181 CAAGCGATGGACAAGTTGTCTGAGTCAGGATTTGCGCAGATGCTCCGTCGAGATCCTCAA 240
GlnAlaMetAspLysLeuSerGluSerGlyPheAlaGlnMetLeuArgArgAspProGln

241 AAAGAGCGAGAAGCCGGTCTGTTTTGCGATATCGCACAGGCCATTCTGCAAAACGGCGAA 300
LysGluArgGluAlaGlyLeuPheCysAspIleAlaGlnAlaIleLeuGlnAsnGlyGlu

301 GCGTATGAACGCGATGCCACGGATGCCTTTCAGGAAGTAGTCAGCGATTTGTACGACGGT 360
AlaTyrGluArgAspAlaThrAspAlaPheGlnGluValValSerAspLeuTyrAspGly

361 TTTTTAAGCGAGGAAGACAGGAGTGGCATCAAACCGCCTGATGAAAGCTTGATTGCTCCT 420
PheLeuSerGluGluAspArgSerGlyIleLysProProAspGluSerLeuIleAlaPro

421 CTGGTCAAATGGGGACGCCCGCAATTCGGACCTTATACGTGGACAGCTGAAGCCGCTGCC 480
LeuValLysTrpGlyArgProGlnPheGlyProTyrThrTrpThrAlaGluAlaAlaAla

481 CATTTTGGCATCAAGACGGGCATTGTCAATTTGCCCCCGGCAAACGCCCGCCTGGGTCTG 540
HisPheGlyIleLysThrGlyIleValAsnLeuProProAlaAsnAlaArgLeuGlyLeu

541 CTCGCGTGGTCTGCATTAGGTCACGAAACGGCTGGACACGACATTCTCCACGCCGACACC 600
LeuAlaTrpSerAlaLeuGlyHisGluThrAlaGlyHisAspIleLeuHisAlaAspThr

601 GGTTTGCTTGGAGAACTGCAGCAAACCGTCTATGACGCTTTGTTTGATGAGCTTCACAAT 660
GlyLeuLeuGlyGluLeuGlnGlnThrValTyrAspAlaLeuPheAspGluLeuHisAsn

661 CGGACGCTGGCGGACTACTGGTCGCTCCGAATCGACGAGACTGCCTCCGACGTTTTGGGA 720
ArgThrLeuAlaAspTyrTrpSerLeuArgIleAspGluThrAlaSerAspValLeuGly

721 ATCCTGAACACCGGCCCCGCTGCAGGGATTGGACTGATTGGATATTTCCGCGGCCTTAAT 780
IleLeuAsnThrGlyProAlaAlaGlyIleGlyLeuIleGlyTyrPheArgGlyLeuAsn

781 AAGGCGTACACCGGACAAGCAACACTGCGGAATACAGGGCCACAGAATGACCCACATCCA 840
LysAlaTyrThrGlyGlnAlaThrLeuArgAsnThrGlyProGlnAsnAspProHisPro

Fig. 13

841 GCAGACATCTTGCGCGGTTATCTTGCTGCTGAGACTGCTCGTCTGCTGCATTTTGACAAC 900
AlaAspIleLeuArgGlyTyrLeuAlaAlaGluThrAlaArgLeuLeuHisPheAspAsn

901 GCATCCGACTGGGCACAGGCACTTCTCGAGGAAACCAGGCGTGATCTTAAAGGCATCACA 960
AlaSerAspTrpAlaGlnAlaLeuLeuGluGluThrArgArgAspLeuLysGlyIleThr

961 ATAGGCAGAGCCTCTTTGGATGCAGAAACCGCTCAAAAATCTGCTGCCATTGTCGCTCGC 1020
IleGlyArgAlaSerLeuAspAlaGluThrAlaGlnLysSerAlaAlaIleValAlaArg

1021 ACAATTATGGAAGCACGCCTGCTCAGTCTGGAAGGTCATGCCCTCGGGCAAATTCAAAAC 1080
ThrIleMetGluAlaArgLeuLeuSerLeuGluGlyHisAlaLeuGlyGlnIleGlnAsn

1081 TGGCACAACGAGGATGAACGAATCGTTCAGGAAATTCGCTCCCATTTTACAGGTTCCCTG 1140
TrpHisAsnGluAspGluArgIleValGlnGluIleArgSerHisPheThrGlySerLeu

1141 ACCGTGCAAGACGGCATTGTTTCGGGTATGTATGCTGCGCATGTCGTGGCAGCAGCCGTC 1200
ThrValGlnAspGlyIleValSerGlyMetTyrAlaAlaHisValValAlaAlaAlaVal

1201 CAAGCAGCCGTTTCAGGAGAGATGGATACCTCCGCTGCCTTCACAGGGATGAAAACCTTG 1260
GlnAlaAlaValSerGlyGluMetAspThrSerAlaAlaPheThrGlyMetLysThrLeu

1261 CTGAAGAGCATGCACGACGCCAATCCTTCCTGGGGACCTCTCTATGTACGATATCGCGGT 1320
LeuLysSerMetHisAspAlaAsnProSerTrpGlyProLeuTyrValArgTyrArgGly

1321 GATCTCACTCCGCATCGCATTTACTCCCGTTCTGCGAGCTAG 1362
AspLeuThrProHisArgIleTyrSerArgSerAlaSer***

Fig.14

PRIMER NAME	OLIGONUCLEOTIDE SEQUENCE
Hos P1	gggggtacctcactctgtcagcatgctg
Hos P2	gggggatcccggcgtgattcccactgc
Hos P3	gggctgcagatagcggatgaaggtgtg
Hos P4	gggtctagacctgcttatacatctgtttcg

Fig.15

PRIMER NAME	OLIGONUCLEOTIDE SEQUENCE
imp P1	gagagaccATGGACCATCCTGATTTTCGCGATCTACCCG
imp P2	agaattcagtggtggtggtggtggtggtgGCTCGCAGAACGGGAGTAAATGCGATGC

Fig. 16

flp P1: aaaagaattctttctgcagaacaggatgcgggggagccgccgct

Fig. 17

flp P2: aaaaaggatccttatagcatctaatcttcaacaaact

Fig. 18

flp P3: aaaaaaagatcttgaacgatgacctctaataattgttaa

Fig. 19

flp P4: aaaagaattcaaatctagaaagtgtgtgctctgcgaggctgtc

Fig. 20

flp P5: tccatggcacaatttggtatattatgtaaa

Fig. 21

flp P6: actcgagttatatgcgtctatttatgtaggat

Fig. 22

flp P7. tttttctagactttatgaatataaagtatagtgtgt

Fig. 23

flp P8: gggggctgcagttatatgcgtctatttatgtaggatg

Fig.24

PRIMER NAME	AMINO ACID SEQUENCE DATA	PRIMER OLIGONUCLEOTIDE SEQUENCE
emp P1	LysArgValHisThrAspAsnLeu	aaRcgIgtNcaYacNgaYaaYct
emp P2	PheGlnThrGlnProThrGlyPhe	aaNccIgtNggYtgNgtYtggaa

I : INOSINE, R : A or G, Y : C or T, N : A or G or T or C

Fig.25

PRIMER NAME	OLIGONUCLEOTIDE SEQUENCE
emp P3	cctcgtagtgcttttggtcgaag
emp P4	accaataccggagtgaaccagca
ADAPTOR PRIMER	actatagggcacgcgtggt

Fig. 26

ctcccatggctttcgctacccccgtgcagtccgtggactgc

Fig. 27

atataagcttttagggagagaggacttccatggt

Fig. 28

tttctgcaggtaaaatcgaagaaggtaaactggta

Fig. 29

aaaaagcttttacttggtgatacgagtctgcgcg

Fig. 30

ttttggatccgaggaggtgtcggagaactgtagccac

Fig. 31

aaaaagcttctacactggcagctcctcctgtctg

Fig. 32

aaggatccccgtcatatccggca

Fig. 33

aaaagctttaggcgttatccgctttagc

Fig. 34

tatatccatggcttcttactgccaggcgcccttttttaa

Fig. 35

atataagcttttattttgatgctctctggccttggaa

Fig. 36

atattcatgagcaacgacttgcttcgatccca

Fig. 37

atataagctttcagttctggagataatctgtaagta